INFLUENCE OF AMORPHOUS CALCIUM PHOSPAHTE ON THE PROPERTIES OF CALCIUM PHOSPHATE BASED CEMENTS

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A thesis submitted in partial fulfillment of the requirements for the degree of

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To

Ceramic Engineering Department

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By

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CERTIFICATE

This is to certify that the project entitled, **"INFLUENCE OF AMORPHOUS CALCIUM PHOSPHATE ON THE PROPERTIES OF CALCIUM PHOSPHTE BASED CEMENT"** submitted by **Shubhashree Sahoo** bearing Roll no. **110CR0107**, is an authentic work carried out by her under my supervision and guidance for the partial fulfillment of the requirements for the award of Bachelor of Technology Degree in Ceramic Engineering at National Institute of Technology, Rourkela.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University or Institute for the award of any Degree or Diploma.

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Date: 10.05.2014

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Contents

ABSTRACT

In the present study, the influence of amorphous calcium phosphate (ACP) on the properties of α- tricalcium phosphate (α-TCP) derived calcium phosphate cement (CPC) was investigated. Properties like setting time, formed apatite crystallite size and diametral tensile strength of ACP/α-TCP based cement was studied. ACP powder was synthesized using rapid wet precipitation method from a precursor solution of calcium nitrate and ammonium dihydrogen phosphate. Synthesis of α-TCP was carried out by the sold reaction between calcium carbonate and dicalcium phosphate anhydride at 1300 C followed by rapid quenching. α- TCP and ACP powder were synthesized phase purity was analysed using x-ray diffraction (XRD) and Fourier transform infra red (FTIR) spectra. Initial and final setting times of the cement pastes were measured at 37 °C in humidity controlled incubator. The phase composition and microstructure of the set cements were investigated after 10 days in SBF using X-ray diffractometry and scanning electron microscopy. Diametral tensile strength of the set CPCs was measured using Universal Testing Machine. The α-TCP-based cement exhibited longer setting times, a higher strength as compared to ACP added CPC. Addition of ACP to the α-TCP based cement resulted in shorter setting times, but larger macroporosity and diminished diametral tensile strength. Moreover, well entangled hydroxyapatite crystals were observed in the microstructure of α-TCP based CPC.

CHAPTER # 1
INTRODUCTION

1. INTRODUCTION

Ceramics have brought a tremendous transformation in human culture from roving hunters to agrarian settlers since thousands of years ago. A recent development has occurred in this field during the last few decades to bring improvement in the quality of human life. The development is to use ceramic materials for the repair and reconstruction of skeletal diseases and disorders. These manmade materials which are used as clinical implants and exhibit specific positive response within the body are called bioceramics. These materials are biocompatible and therefore have many clinical applications for instance, repair of the skeletal systems, to supplement both types of tissues, hard and soft and to replace fragments of cardiovascular system.

Many improvements are being carried out in medical facilities which has increased the life expectancy of human beings. With this the requirement for advanced biomaterials to check bone damages and dental complications is gaining speed in growth mostly for aging population. There is a far way to go in this field for developing and fabricating new materials which can imitate both structures and functions of natural tissues.

Compounds of calcium phosphate, because of their chemical composition, satisfy the conditions of biocompatibility in an exemplary manner. The vital components of these materials dismiss the immunological defense reactions and dangerous actions. The calcium-phosphate based compounds such as tricaicium phosphate, tetracalcium phosphate and synthetic hydroxyapatite are imperative for implantation.

Calcium phosphate cement (CPC) is a hydraulic cement. It is formed by a mixture of more than one calcium orthophosphate minerals. Calcium orthophosphate powder has been in research for the last 80 years as bone repair material. Tricalcium phosphate based cement especially alpha $Ca_3(PO_4)_2$ (α-TCP) , a type of CPC, is an emergent raw material for numerous injectable bone cements, bioceramics and composites for bone restoration.

Precipitated hydroxyapatite (HA) or brushite (DCPD) are two different end products of CPCs. The best stable calcium phosphate is HA at pH>4.2 and DCPD at pH<4.2. Finished product of the setting reaction of apatite cement formulations is precipitated poorly crystalline

hydroxyapatite (HA) and/or calcium deficient hydroxyapatite (CDHA). These reactions are carried out in an aqueous milieu and have less crystallinity. These are similar with the mineral phase of bones and teeth. These properties are responsible for their outstanding *in vivo* resorption characteristics.

Brushite cements have DCPD as their main end-product of the setting reaction which is an acid-base reaction. The brushite cement paste is always acidic throughout setting. More the acidic nature easier and faster will be the preparation, better will be the reactivity, and there will be better physico-chemical properties, such as slower setting times and greater mechanical strengths due to a higher homogeneity. But, more acidic environment results in degradation of biocompatibility of the cement preparation, because of low pH values at the time of setting.

CPCs can either be monocomponent or multicomponent. In monocomponent CPCs, a single calcium-phosphate compound such as alpha tricalcium phosphate (α-TCP) after hydrolysation converts to CDHA according to Eq. 1 without changing the Ca/P ratio

$$3\alpha\text{-}Ca_3(PO_4)_2 + H_2O \longrightarrow Ca_9(HPO_4)(PO_4)5OH \quad (1)$$

In multicomponent CPCs, set following an acid–base reaction.

Apatitic cement: $$2Ca_4(PO_4)_2O + 2CaHPO_4 \longrightarrow Ca_{10}(PO_4)_6(OH)_2 \quad (2)$$

Brushitic cement: $$\beta\text{-}Ca_3(PO_4)_2 + Ca(H_2PO_4)_2.H_2O + 7H_2O \longrightarrow 4CaHPO_4.2H_2O \quad (3)$$

α-TCP sets as a result of the precipitation of an entanglement of apatite crystals according to Eq.1 mentioned above. The resulting calcium deficient hydroxyapatite (CDHA) is similar to bone material but is partially soluble in physiological states and the bioresorption and osteotrasduction are very sluggish. As a result, after months of implantation, considerable amount of the cement remains. Furthermore, the slow setting reaction and the low strength, especially after the initial setting time are not ideal for clinical applications. To overcome this problem, addition of calcium phosphates of high reactivity obtained by decreasing the size of particles and crystallinity, can be made.

By adding amorphous calcium phosphate (ACP) to the preparation could improve the bioresorbability of the end product and the setting reactions. The long range periodic atomic scale arrangement of crystalline HAp is deficient in precipitated ACP, has a high specific surface area. Moreover, ACP based cement formulation displays negligible significant hardening at room temperature, does harden very rapidly at body temperatures. Such endothermic setting behavior is beneficial, from clinical point of view, as it extends the working time at room temperature and guarantees a quick setting after implantation.

Hence, the aim of this study was to observe any change in the setting time, the strength and microstructure by adding ACP to cement formulation and comparing it with the formulation not containing ACP.

CHAPTER # 2
LITERATURE REVIEW

2. LITERATURE REVIEW

The advancement of recent bone replacing materials has drawn academic and commercial interest for the need for a synthetic bone replacement that meets the drawbacks of current orthopedic and dental methods. The vital disadvantage of these methods is not adapting to the accurate shape of the defect. Which results in improper contact between the implant and surrounding tissues [1]. Amongst several ceramics which have been examined for their use inside the human body, calcium phosphate based ceramics have gained the most interest because of their superior biocompatibility and compositional similarities to bone. Presently, these ceramics are used as coatings, powders, and in particulate forms and are used in repair of maxillofacial and dental defects, drug delivery and as bone filler [2].

Calcium phosphate-based biomaterials is the most frequently studied synthetic bone graft since 20 years and particularly the self-setting CPCs. CPCs form a mouldable paste when an aqueous liquid is added. The paste sets in situ by forming apatite crystallites that are similar to the mineral phase of bone [3, 4]. So, it is clear that CPCs are not only biocompatible with bone and the soft tissues surrounding the bone, but also shows bioactivity, osteoconductivity and can induce osteointegration [5-7]. The distinctive advantage of CPCs is that they can very well adapt to the surfaces of tissues in a defect.

Self-setting calcium orthophosphate formulations are prepared by mixing amorphous and/or crystalline calcium orthophosphate powder(s) with an aqueous solution for instance, distilled water, phosphate-buffered saline (PBS), aqueous solutions of sodium orthophosphate, orthophosphoric acid, citric acid, sodium silicate, magnesium hydroorthophosphate or simulated body fluid (rSBF). Within a few minutes of mixing the powder(s) and the solution, a viscous and moldable paste is formed that sets to a firm mass. When the paste becomes satisfactorily stiff, it is introduced into a defect, where it hardens in situ within the operating theatre.

CPCs set as a result of a dissolution and precipitation process. The entanglement of the precipitated crystals is responsible for cement hardening unlike acrylic bone cements, which harden due to polymerization reaction

Brown and Chow introduced hydraulic CaP cements by mixing tetracalcium phosphate and dicalcium phosphate [8]. A range of CaP materials has now been investigated [9-11]. One of the more promising of these CaP materials is alpha phase tricalcium phosphate (α-TCP). Hydration of α-TCP powders with water or soluble phosphates in solution leads to the dissolution of calcium phosphate and deposition of a more stable, lower energy form of calcium phosphate, calcium- deficient hydroxyapatite (CDHA) [12, 13]. But the disadvantages are, less solubility in physiological conditions, slow bioresorption and osteotransduction, sluggish setting reaction is not desirable for clinical application.

Some experiments have been carried out taking the fast resorbing CPC based on ACP and dicalcium phosphate dihydrate (DCPD) [14-16]. This cement has nanocrystalline apatitic end product and gets nearly fully resorbed and replaced by new bone after 26 weeks of establishment during the hardening process. Such fast characteristic, is mostly due to the effect of the large specific surface area of the nanocrystalline apatitic product. The large specific surface area not only causes a fast resorption of the cement, but also enhances osteoblast precursor cell attachment through the amount of protein adsorbed [17, 18]. It is studied that, addition of ACP in calcium phosphate based cement formulation, enhances the setting reactions in the system, as a result, at room temperature, working time is enhanced but decrease in setting time is observed after implantation.

CHAPTER # 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Good grade chemicals and ultrapure water were used throughout the preparation of the solutions and the constituents of the cement powders.

3.1. Synthesis of Amorphous Calcium Phosphate

3.1.1. Wet route synthesis

To synthesize ACP powder wet chemical precipitation route was followed. This route followed double decomposition of calcium and phosphate salts in water-alcohol solutions at ambient temperature and at pH close to 9. Calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$ and di-ammonium hydrogen phosphate $(NH_4)2HPO_4$ were used as a source of Ca ad P respectively.

6.945 g of $Ca(NO_3)_2.4H_2O$ and 0.15 g (2 mol% of Ca(NO3)2.4H2O taken) of magnesium nitrate hexahydrate $Mg(NO_3)_2.6H_2O$ were put in ethanol. $Mg(NO_3)_2.6H_2O$ was put in the calcium nitrate solution to inhibit the conversion of amorphous form of calcium phosphate to its crystalline form. 4.08 g of $(NH_4)2HPO_4$ was dissolved in very small amount of water (because $(NH_4)_2HPO_4$ is sparingly soluble in ethanol). NH_3 solution was added drop wise to this solution in order to maintain the pH to 9. As soon as the two solutions were mixed rapidly, white gelatinous precipitate appeared which was stirred for few seconds and processed as follows.

3.1.2. Washing

The precipitate was washed thoroughly with distilled water by centrifugal process using centrifuging machine. The speed of the machine was set to 8000 rpm and it was run for 3 minutes. The sample was washed in order to remove Mg^{2+} ions present in the precipitate. The washing procedure was done only once to check the conversion of amorphous form of ACP to crystalline form due to several washing cycles. The reason behind this was the direct contact of precipitate with moisture while carrying out the washing procedure that helps the conversion. The washed precipitate was then stored in a freezer; prior requirement of freeze drying.

3.1.3. Freeze Drying

The frozen precipitate was then kept in a freeze dryer for freeze drying. Freeze drying was done instead of oven drying to get agglomerated free powder. In oven drying, the liquid present inside a powder would bring particles close to each other due to the force of surface tension and result in agglomeration. In freeze drying, the sample was first frozen to the temperature below the triple point. The surrounding pressure was then reduced by applying vacuum to allow the ice surrounding the powder to sublimate directly from solid phase to gas phase to get agglomerate free powder with uniform particle size distribution. The freeze dried sample was then kept in freeze until use.

These processes were repeated several times for the synthesis of ACP, but each time instead of amorphous phase, crystalline phase appeared due to the formation of hydroxyapatite. All the powders were preserved for the future use. In the last attempt, after the characterization of powder, it was concluded that the amorphous phase appeared and powder was correctly formed. Powder which was the "converted hydroxyapatite" was also used in the study as by incorporating it in cement formulation and observing any property change on its addition in α-TCP based cement.

3.2. Synthesis of α-TCP Powder from DCPD

Dry route synthesis was followed for preparing α-TCP powder. In this route, di-calcium phosphate dihydrate (DCPD) was first converted to di-calcium phosphate anhydrous (DCPA) by heating it in a furnace at 180^{o}C for 4 hours which removed the bounded water. The above conversion was made by taking 35 g of DCPD according to the stoichiometric calculations. 27.2 g of the formed DCPA was taken and mixed with 10 g of calcium carbonate $CaCO_3$ powder. The mixture of DCPA and $CaCO_3$ was milled for 13-15 hours in a pot mill to ensure proper mixing of the two fine powders. Propanol was used as a wetting medium for the mixture. The milled mixture was dried in an oven for 24 hours at 60^{o}C and then kept in a raising hearth furnace for firing at 1300^{o}C. After 6 hours of soaking time, the sample was air quenched. The α-TCP reagent was powdered and sieved to obtain 38 πm sized powder.

3.3. Preparation of Simulated Body Fluid (SBF) solution

SBF is a metastable buffer solution [19, 20], and even a small alteration in preparation step and the temperatures, may greatly alter the purity of phase of the soaked samples. Pure quality NaCl (99.5%), $NaHCO_3$ (99.5%), KCl (99.0%), $Na_2HPO_4.2H_2O$ (99.5%), $MgCl_2.6H_2O$ (99.0%), Na_2SO_4, $(CH_2OH)_3CNH_2$ (99.5%), $CaCl_2.H_2O$ (99.0%) and HCl (37 vol%) were used in making SBF for this study. The solution was prepared by adding and dissolving suitable quantities of the desired chemicals listed in Table.1 in distilled water. Chemicals were added, one by one after each chemical was entirely dissolved in 700 ml of water. The addition was according to the order provided in Table 1. pH adjustments was done by adding 40 ml of 1 M HCl in 1 l of SBF solutions. It was taken care that, 15 ml of this acid solution was added just before the addition of the sixth chemical, namely, $CaCl_2.2H_2O$. Or else, the solution become turbid. And the rest part of the HCl solution was used for further titration. The temperature of the solution kept on a stirrer was raised from ambient to 37ºC after the addition of the eighth chemical (tris(hy- droxymethyl)aminomethane). Then 1 M HCl was used to titrate the solution to bring down the pH to 7.4 at 37ºC. At the time of titration, the strength of the solution was constantly weakened with consecutive additions of distilled water to make the final volume equal to 1 l. It was then stored in freeze. Table.1. shows the names of the chemicals for the preparation of SBF solution in the order of addition.

Table 1: Chemical reagents to be used for preparing SBF

Order	Chemicals	Amount (g/l)
i.	NaCl	6.546
ii.	$NaHCO_3$	2.269
iii.	KCl	0.374
iv.	$Na_2HPO_4.2H_2O$	0.177
v.	$MgCl_2.6H_2O$	0.306
vi.	$CaCl_2.2H_2O$	0.367
vii.	Na_2SO_4	0.070
viii.	$(CH_2OH)_3CNH_2$	6.058

3.4. Preparation of Cement Paste

The cement powders were mixed properly by taking the respective ingredients in an agate mortar. Cement pastes, for all formulations, were made by mixing the liquid and cement powder in the ratio of 1:3 for 2 minutes in a small agate mortar with an agate pestle that yielded a workable paste. The liquid used was disodium hydrogen phosphate (Na_2HPO_4) (as this liquid helps in enhancing the apatite precipitation because of the the presence of HPO_4^{2-} [1]) Cement systems that were prepared and studied are tabled below in Table.2. After proper mixing, the paste was set in a mold in the form of tablets. Initial and final setting times were determined at room temperature using light and heavy Vicat needles respectively [21]. After measuring final setting time, the cement tablets were soaked in SBF at 37 °C for 10 days. For further characterization, the samples were used after drying.

Table 2: Cement Formulations of the three samples

Sample No.	Formulation	Weight of Powder (g)	Weight of Liquid (ml)
1	α-TCP	4.5	1.5
2	α-TCP+ 10%HAp	3+0.3	1.1
3	α-TCP+ 10% ACP	3+0.3	1.1

3.5. Characterization

As-synthesized powders (α-TCP and ACP) and set cements of three formulations were characterized using the following techniques.

3.5.1. Characterization of synthesized powders

3.5.1.1. XRD Analysis

The XRD analysis of the synthesized powders (α-TCP, ACP, Hap) was done using Philips X-Ray diffractometer (PW 1730, Holland) with nickel filtered Cu Kα radiation (λ = 1.5406 A°) at

40 kV and 30mA and diffraction patterns were recorded over Bragg's angle 2θ range of 10-80^{o} at a rate of 5^{o}/minute.

3.5.1.2. FTIR Spectroscopy

Fourier transform infrared spectroscopy (FTIR) is a method that is used to get an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of any type of sample, a solid, liquid or gas. A beam of infrared light is passed through the sample, the infrared spectrum of a sample is recorded. Absorption occurs only when the frequency of the IR matches with the vibrational frequency of a bond. Analysis of the transmitted light gives an indication of the amount of energy absorbed at each frequency (or wavelength). This can be achieved by monochromator. The entire wavelength range is calculated at once using a Fourier transform instrument and then a transmittance or absorbance spectrum is generated according to a correct procedure. Analysis of the position, shape and intensity of peaks in this spectrum shows us the details about the molecular structure of the sample. An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. The beam is changed to contain a different combination of frequencies, giving a second data point and this process is repeated many times. Later, a computer takes all this data and works backwards to infer what the absorption is at each wavelength.

Very small amount of powders were used to do this experiment. All the data were taken in the range of 400-4000 cm^{-1}.

3.5.1.3. Particle Size Distribution (PSD)

PSD is an indication showing what sizes of particles are present in what proportions in the sample particle group to be measured. The PSD is crucial in interpreting the physical and chemical properties of a material. It affects the strength and load-bearing properties of materials. It also affects the reactivity of solids participating in chemical reactions, and needs to be tightly controlled in many industrial products such as the manufacture of printer toner and cosmetics.

Particle size distribution profile of small particles in suspension was obtained by the technique of dynamic light scattering. Approximately, 0.002 g of each powder (α-TCP, ACP and HAp) was

added to different beakers containing 50 ml of distilled water and ultra-sonicated for 15 minutes to lessen the degree of flocculation. The Stokes–Einstein equation: $r = kT/6D\pi\eta$ was used to determine the particle sizes where r is the particle radius, k is the Boltzmann constant, T is the absolute temperature, D is the diffusion coefficient, and η is the viscosity of the liquid in which the particles were suspended.

3.5.2. Characterization of set cements

3.5.2.1. Initial and Final Setting Time

The Vicat device was used to measure the setting times of the set cements. The device comprised of a Vicat mold, a metallic frame supporting a freely movable rod with a cap at top, split type and glass base plate and one set of needles one each for initial and final setting time and consistency plunger. The cement paste after set in two molds of height 2 cm and diameter 1 cm each, were characterized for the initial and final setting times using the needles of the device.

3.5.2.2. Scanning Electron Microscopy (SEM)

This technique is used to produce pictures of a sample by scanning it with a beam of electrons. The electrons intermingle with atoms inside the sample, resulting in numerous signals that can be spotted. Those signals contain data about the topography and composition of sample. A sample used for scanning must be of a suitable dimension to match with that of the dimension of specimen chamber. They are mounted tightly on a specimen holder called a specimen stub. In normal imaging, specimen surface is made electrically conductive, and electrically grounded to prevent the accumulation of electrostatic charge at the surface. Metal specimen needs very little exclusive preparation for SEM except for cleaning and mounting the sample on a specimen stub. While, in nonconductive specimens, they get charged up when scanned by the electron beam, and due to this, many scanning faults and other image artifacts are caused. To overcome this, they are coated with an ultrathin coating of electrically conducting material, deposited on the sample either by low-vacuum sputter coating or by high-vacuum evaporation.

3.5.2.3. Diametral Tensile Strength

The diameter and thickness of the set cement pellets were measured. Universal Testing Machine (UTM) was used to measure the compressive strength of the pellets. The pellets were kept on their width and a constant force was applied on the sample. The instrument measures the diametric tensile strength (DTS). DTS is given by the formula:

$$F=2\sigma/\pi dt$$

Where σ is the maximum load at fracture, d is diameter of the pellet and t is the thickness of the pellet.

3.5.2.4. XRD Analysis

The set cement pellets were analyzed for X-ray diffraction patterns obtained using Philips X-Ray diffractometer (PW 1730, Holland) with nickel filtered Cu Kα radiation (λ = 1.5406 A°) at 40 kV and 30mA and diffraction patterns were recorded over Bragg's angle 2θ range of 10-80o at a rate of 5º/minute. The pellets were crushed to powder and taken for the analysis.

CHAPTER # 4
RESULTS
AND DISCUSSION

4. RESULTS AND DISCUSSIONS

4.1. RESULTS

4.1.1. Phase identification of the synthesized powders:

The synthesized powders were analyzed for their XRD patterns. A broad peak in the analysis of diffractograms of ACP powder signified the presence of an amorphous phase. This is shown in Fig.1. Some degree of crystallinity in the XRD pattern may be because of partial hydrolysis of highly susceptible ACP nanocrystals into HA phase on exposure to moisture.

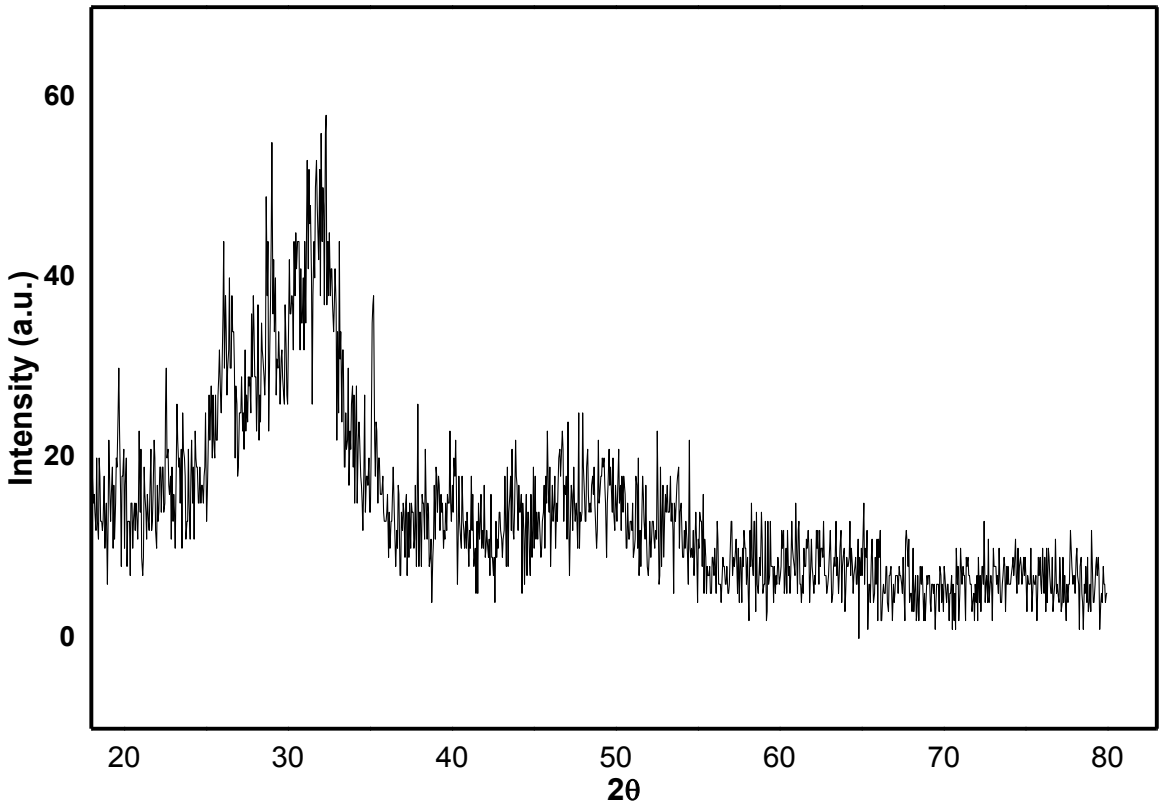

Figure 1: X-ray diffractogram of the as- synthesized ACP powder

The XRD pattern of synthesized α-TCP powders is shown in Fig.2. All the major peaks was matched with that of the JCPDS powder diffraction data file 03-0690 which revealed the fact that the synthesized α-TCP powder was almost phase pure.

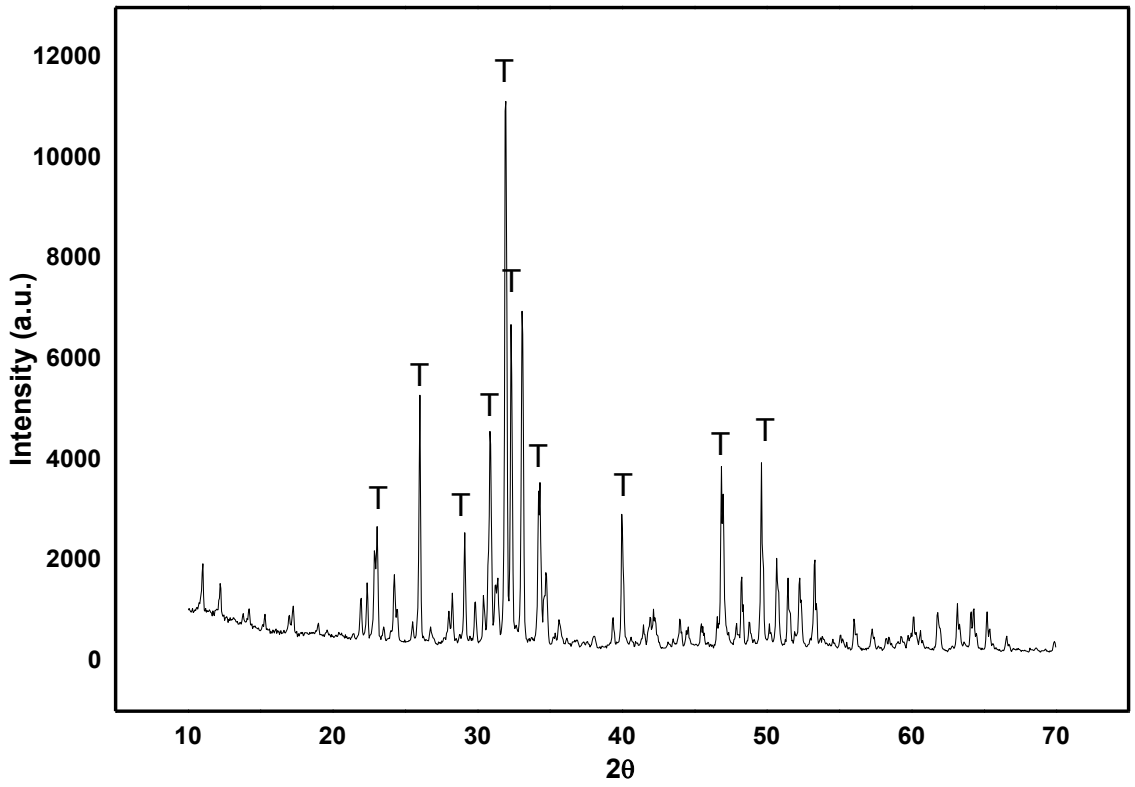

Figure 2: X-ray diffractogram of as-synthesized α-TCP powder (T signifies α-TCP)

4.1.2. FTIR Analysis

FTIR analysis of synthesized α-TCP powders is shown in figure3. The IR spectra reveal typical absorption bands at 568 and 605 cm^{-1} due to the ν_4 vibration components of PO_4^{3-} in TCP. Absorption band at 970, 1045 and 1100 cm^{-1} was due to ν_1 and ν_3 stretching of PO_4^{3-} groups. The absence of characteristic absorption due to the stretching of OH^- groups confirmed that the no HA phase was present in the synthesized α-TCP powder.

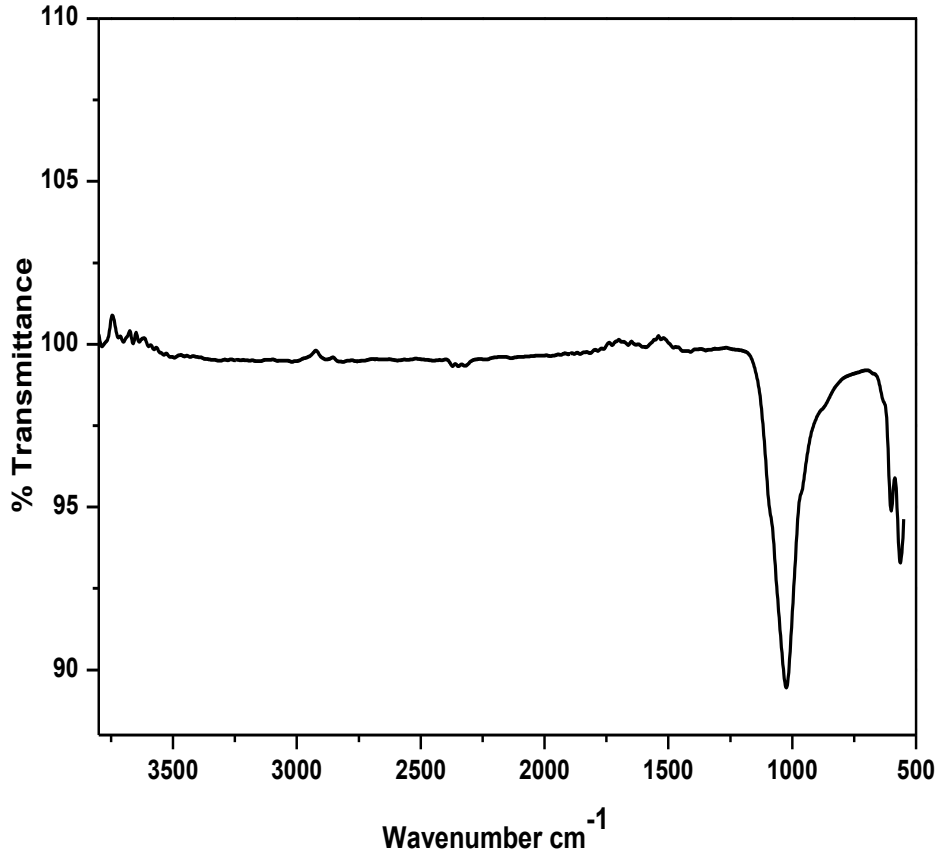

Figure 3: IR spectrum of synthesized α-TCP powder

4.1.3. Particle Size Distribution of the powder sample:

The particle size distribution plots are shown from Fig.4 to Fig.6. The average particle size values of all the synthesized powders were obtained from both intensity and volume percent distribution of the particle size and are tabulated in Table 3. α-TCP showed the highest average particle size of 1.13 μm whereas ACP showed the lowest average particle size of 349.42 nm. The synthesized ACP powders showed the narrower particle size distribution between 250 nm- 360 nm which renders it almost monodisperse.

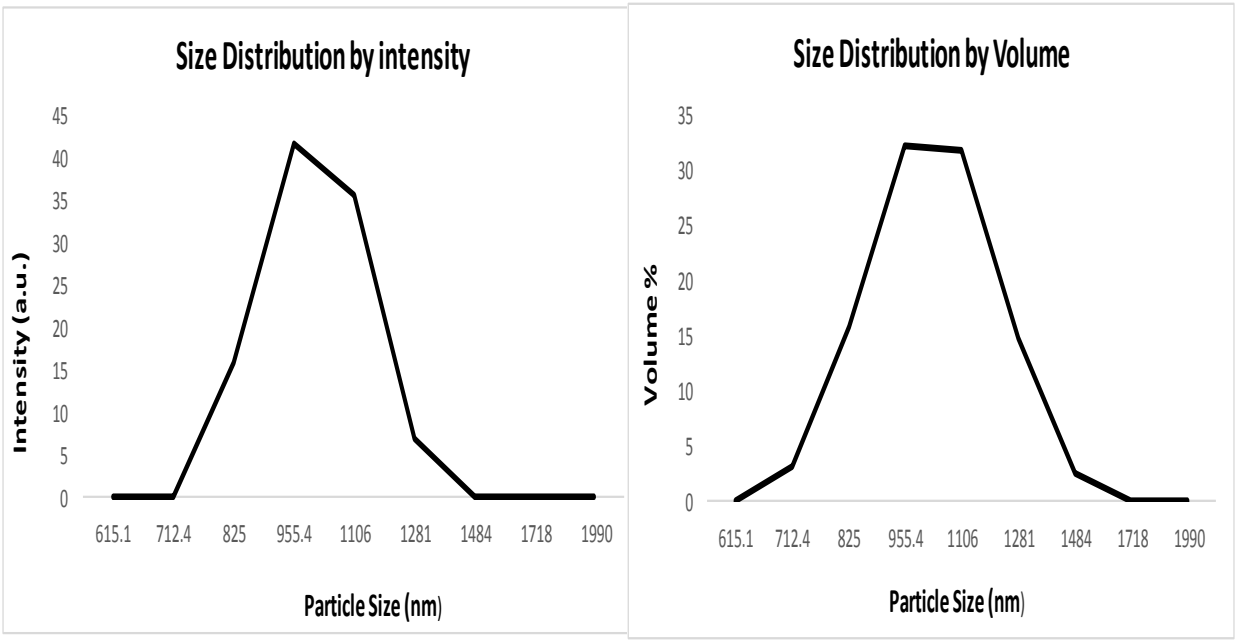

Figure 4: Particle size distribution plot for synthesized *α*-TCP powder

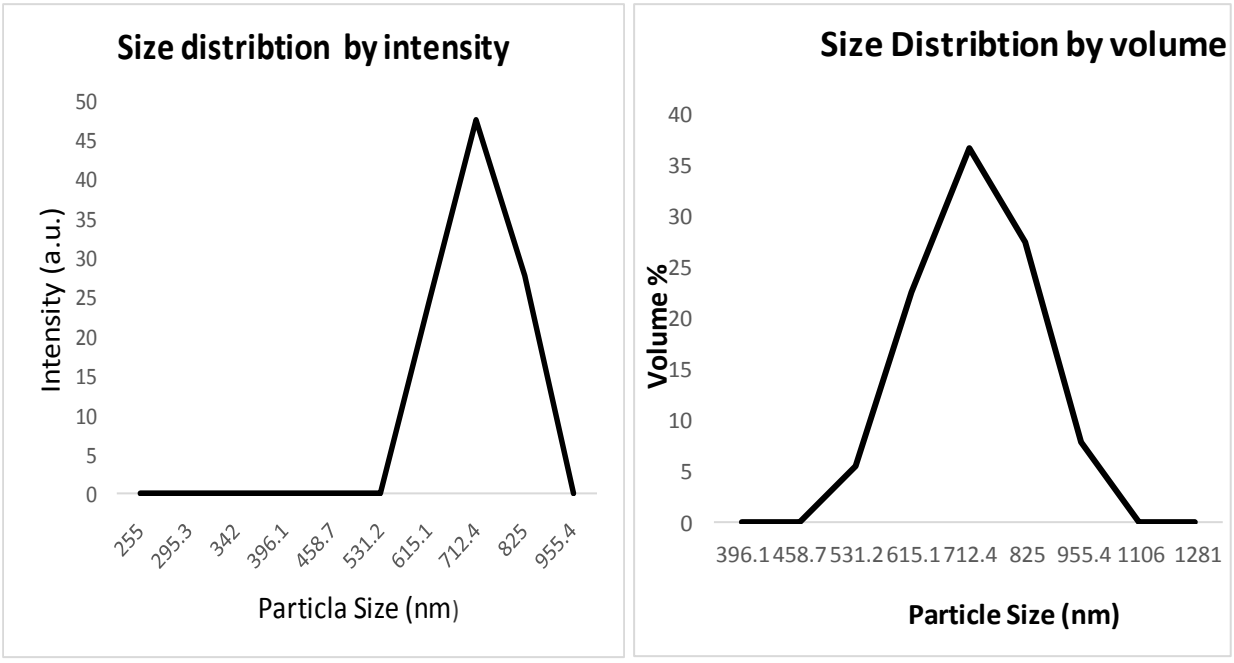

Figure 5: Particle size distribution plot for Hap powder

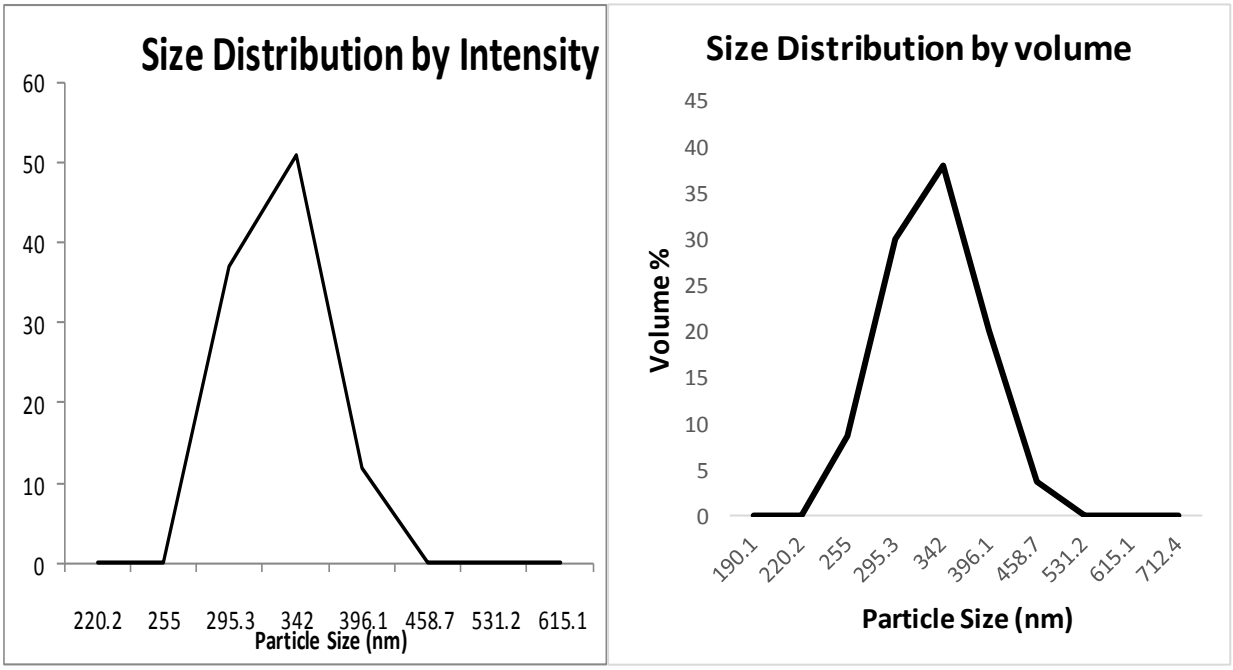

Figure 6: Particle size distribution plot for synthesized ACP powder

Table 3: Average particle size values of a-TCP, HAp and ACP

Sample	Avg. Particle Size (nm)
α-TCP	1130.25
HAp	727.8
ACP	349.42

4.1.4. Initial and Final Setting Time of the Set Cement Pellets

Sample No.	Initial Setting Time (min)	Final Setting Time (min)
1	19	70
3	11	45

Table 4: Initial and final setting times of the set cement pellets

The results of the initial and final setting time of the set cement pellets are given in Table.4.

Sample one, which is the α-TCP based cement showed longer setting times than that of the α-TCP/ACP based cement. Addition of ACP to α-TCP significantly improved both the initial and final setting times by enhancing the setting reactions during hardening.

4.1.5. Microstructure

From the micrograph given in Fig. 7 needle like crystals in all directions could be seen. Blade-like structures were observed between these crystals. On adding ACP to α-TCP, needle-like crystals existed, but these were considerably smaller compared to those detected for α-TCP-based cement. Blade-like was absent. This can be observed in Fig.9. Also, from the microstructure it is evident that the ACP added CPC (Fig. 9) showed higher amount of macroporosity and lower compactness as compared to pure α-TCP based CPC (Fig.7).

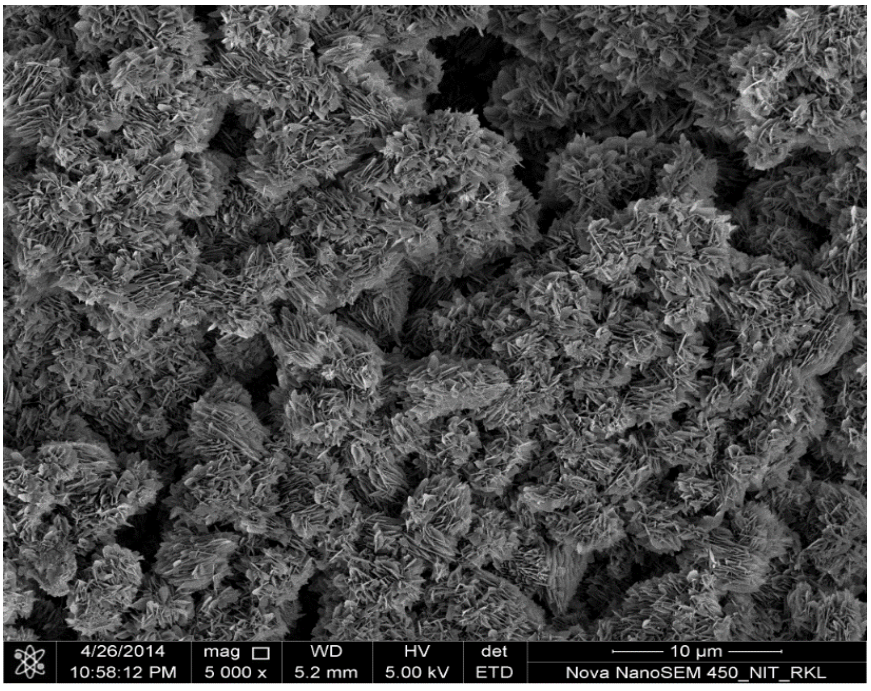

Figure 7: SEM micrograph for α-TCP based set cement

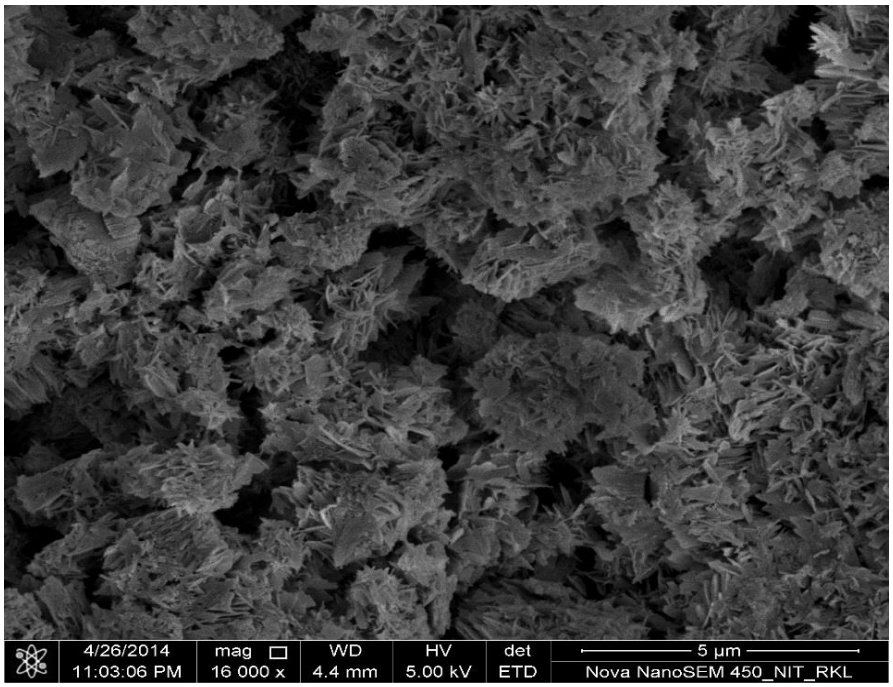

Figure 8: SEM micrograph of HAp based set cement

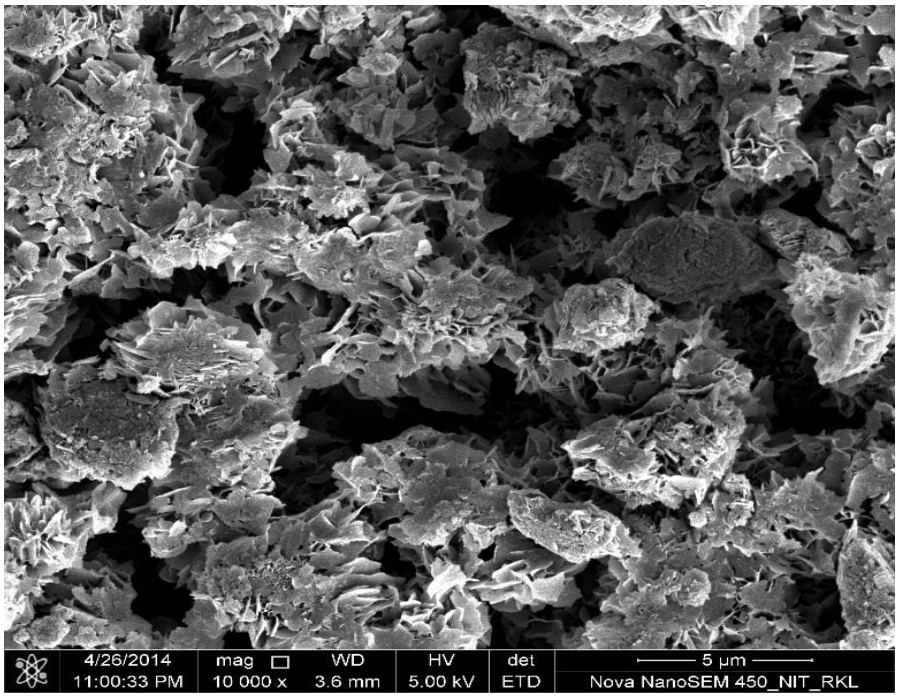

Figure 9: SEM micrograph of ACP based set cement

4.1.6. Phase Analysis of the Set Cement Pellets

In Fig.10 comparison of the X-ray diffractograms of the set cements of three formulations are shown. In all the samples, after keeping them in SBF solution for 10 days at 37^{o}C, a transformation to apatite phase was observed. The mai peaks could be assigned to apatite on the foundation of the JCPDS powder diffraction data file 9-0432.

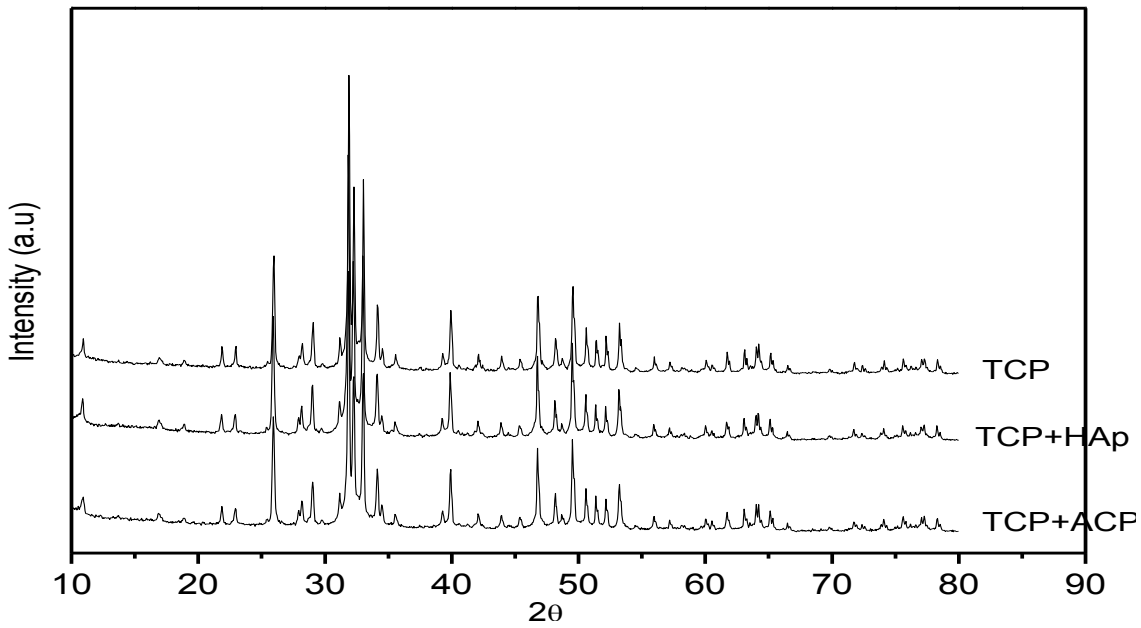

Figure 10: XRD diffractogram of the reaction products of the CPCs after soaking for 10 days in SBF

4.1.7. Diametral tensile Strength

The plots of DTS are shown below from Fig.11 to Fig.13. Table.5 shows the diameter and width of the set cement pellets and the maximum load at fracture. α-TCP derived cement showed higher strength than that when ACP or Hap was added to α-TCP. Maximum load at fracture for α-TCP based cement is 72.33 N which is much more than ACP/α-TCP based cement or Hap/α-TCP based cement, 12 N and 17.33 N respectively. Thus pure α-TCP based CPC showed a DTS value of 1.20 MPa after 10 days of immersion in SBF which is way higher than that showed by HAp and ACP added CPC.

Table 5: Dimensions and maximum load at fracture of the set cement pellets

Sample	Diameter (mm)	Width (mm)	Maximum Load (N)
1	8.84	2.81	72.33
2	8.82	2.89	17.33
3	8.98	2.92	12

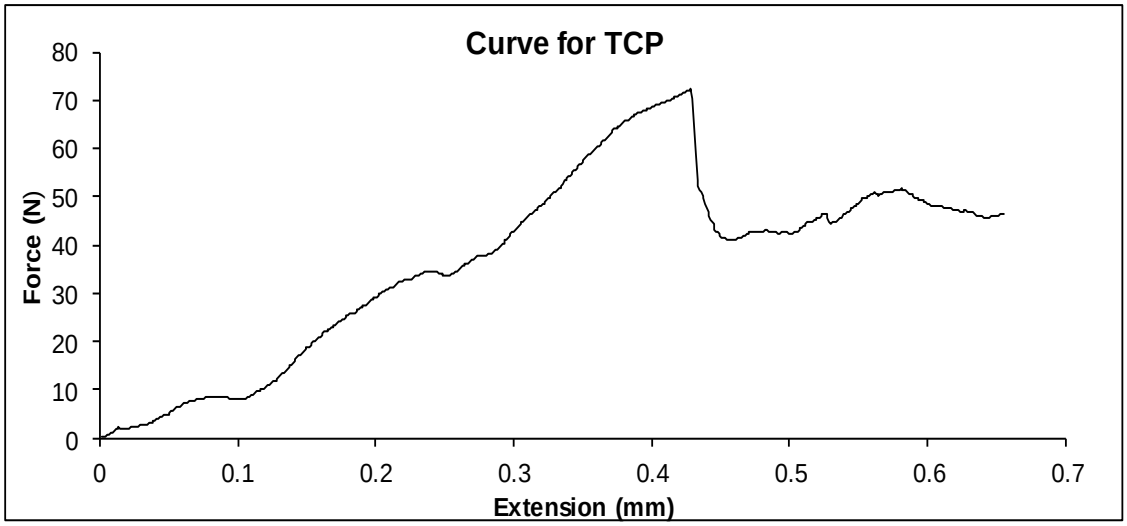

Figure 11: Load vs extension curve for TCP added CPC

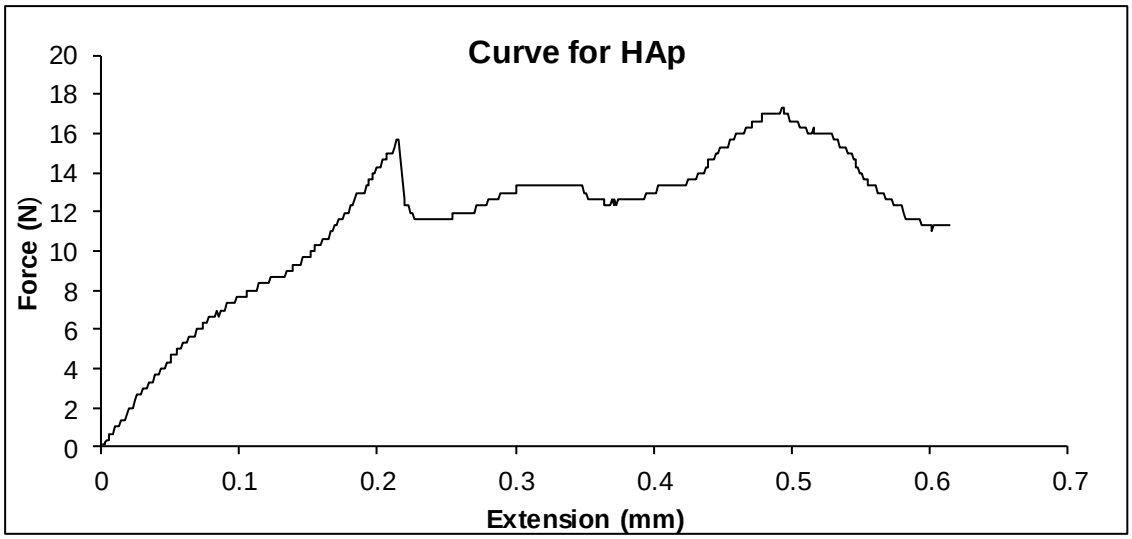

Figure 12: Load vs extension curve for HAp added CPC

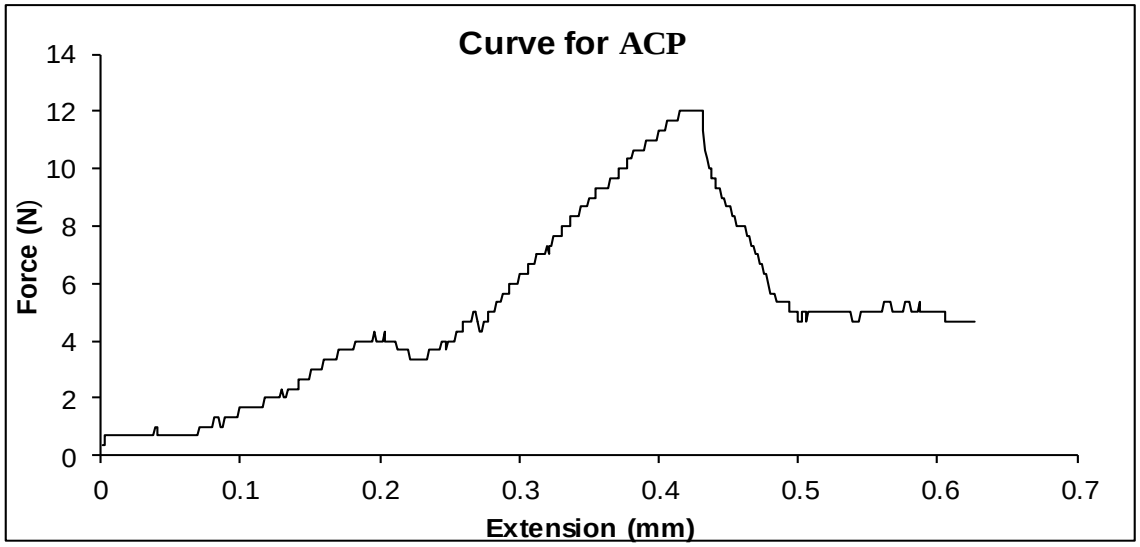

Figure 13: Load vs extension curve for ACP added CPC

4.2. DISCUSSIONS

When α-TCP transforms to an apatite phase, the crystals gradually become entangled, causing the cement paste to get set and provides rigidity and strength to the set cement. In the current study, the initial setting of the CPC formulations was exhibited at only room temperature. At the time of operation, such cements need to be moulded and applied to the desired site before reaching the initial setting time at room temperature. After implantation, final setting inside body should occur rapidly.

The setting times of the α-TCP-based cement drastically decreased at room temperature by supplementing it by adding ACP. This is in accord with the requirement for clinical applications. The setting times decreased because of the formation of nanometer-sized apatite crystals at the time conversion of ACP. These assist heterogeneous nucleation by providing effective sites and accelerate apatite precipitation which in turn promote cement setting [22].

The strength of α-TCP based cement also decreased drastically on addition of ACP. Addition of ACP accelerated the setting reactions of cement primarily because of its higher solubility in SBF supplying necessary Ca^{2+} and PO_4^{3-} ions for nucleation and growth of HAp crystals. So the initial reaction with ACP addition becomes favorable for hardening of CPC but diametric tensile strength showed that addition of ACP hampered the strength of CPC. ACP being spherical in

nature gives high compactness in themselves but fails to produce sufficient amount of elongated and acicular HAp crystals favorable for crystal entanglement for CPC grains. As a result CPC resulted from ACP addition showed larger amont of macroporosity and less compactness and hence lower DTS as compared to pure CPC derived from α-TCP.

Adding ACP to the α-TCP cements brings fluctuation in the microstructure and crystal sizes making them small in size. The formed ACP/α-TCP-based cement that is set, is constituted of nano-sized needle-like particles. Apparently, the presence of significant amount of ACP, results in higher amount of the apatite nuclei, limiting significant growth of the crystals. The modifiaction in microstructure and the smaller crystal size of the set cements due to the addendum drastically reduce the mechanical strength and limit their uses to non-load bearing applications.

CHAPTER # 5
CONCLUSION

5. CONCLUSIONS

The α-TCP-based cement transformed to CDHA comprised of needle-like crystals and blade-like structures. Diametral tensile strength of α-TCP derived cement was higher but the setting reaction was not suitable for medical applications. On adding ACP to α-TCP based cement, the crystallinity of the end product of the setting reactions was decreased and the setting time of CPC was arrested, but the resulting nanocrystalline apatite in CPC were spherical in nature and did not entangle properly. Furthermore, the presence of ACP in CPC resulted in more macroporosity in microstrcture and hence diminished mechanical strengths. As a result, ACP/α-TCP showed apposite setting times, displayed diminished strengths appropriate for non-load-bearing applications. Therefore, these cements are tremendously helpful for bone substitute materials like in, for instance, cranioplasty operation. But, more studies are required to enhance the mechanical strength of APC added CPC without compromising on its favorable setting behavior.

CHAPTER # 6

REFERENCES

6. REFERENCES

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